Title: Impact of tank background on the welfare of the African clawed frog, *Xenopus laevis* (Daudin)

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Abstract:

The captive environment of a laboratory animal can profoundly influence its welfare and the scientific validity of research produced. The African clawed frog (*Xenopus laevis*) is a common model organism, however current husbandry guidelines lack supporting quantitative evidence. The visual environment is a fundamental aspect of a captive animal's housing and may affect a number of physiological and behavioural responses. This is particularly important for species such as *X. laevis* where cryptic camouflage is a fundamental defence mechanism. Here male (n = 16) and female (n = 20) *X. laevis* were housed in tanks with ecologically relevant (black) and non-relevant (white) background colours and physiological and behavioural responses observed. Higher levels of water-borne corticosterone were observed in tanks with a white background compared to a black background in females ($p = 0.047$). Increased atypical active behaviours (Swimming: $p = 0.042$; Walling: $p = 0.042$) and a greater degree of body mass loss ($p < 0.001$) were also observed in the white background condition. Together these responses are indicative of increased stress of *X. laevis* when housed in tanks with a non-ecologically relevant background compared to an ecologically relevant background and suggest refined tank background colour may improve welfare in this species.

Keywords: Animal Welfare; Enrichment; Xenopus; Behaviour; Corticosterone; Tank Background.

1 Introduction

The housing environment of a captive animal can profoundly influence its welfare, potentially impacting upon the purpose of captivity (e.g. research, production or conservation). In laboratory species poor physical and mental state can reduce the reliability and repeatability of the scientific results obtained (Poole, 1997). Refinement of the housing requirements of laboratory species is therefore crucial for high quality research and may also result in a reduction in numbers of laboratory animals required (one of the 3Rs). Consequently, much work has investigated suitable housing conditions for most model species within the captive environment (e.g. Olsson and Dahlborn, 2002; Baumans, 2005). Despite this, research into the welfare of model amphibian species remains limited.

The visual environment is an integral aspect of a captive animal's housing. Refined cage/tank colour may improve an animal's visual perception of food items (Strand et al., 2007; Gonzalez-Bernal et al., 2011) and internal cage/tank architecture (Jones and Kaiser, 2005), influence own body colour (Höglund et al., 2002), and provide an increased sense of security in species that rely on crypsis as a means of predator avoidance (Wente and Phillips, 2005). As a result cage/tank colour has been shown to negatively impact on animal growth (e.g. Hilken et al., 1995; Downing and Litvak, 1999), development (Cobcroft et al., 2012), feeding (e.g. Sherwin and Glen, 2003; Rahnama et al., 2015), immunosuppression (Eslamloo et al., 2015), mortality (e.g. El-Sayed and El-Ghobashy, 2011; Sykes et al., 2011; Ikhwanuddin et al., 2012), and behaviour (e.g. Höglund et al., 2002; Cobcroft et al., 2012) in a range of taxa. Cage/tank colour has also influenced behaviour observed during a common laboratory behavioural assay and may therefore have implications on research validity (Sherwin and Glen, 2003). Consideration of cage/tank colour is therefore of upmost importance for both animal welfare and the purpose of captivity, including improved scientific validity.

Xenopus laevis (Daudin) is a common model laboratory species in developmental and genetic research, and 6,379 scientific procedures were performed during 2013 on *X. laevis* in the UK alone (Home Office, 2014). Despite its widespread usage, quantitative evidence for optimal care of *X. laevis* in captivity is sorely lacking (Reed, 2005). *X. laevis* are fully aquatic and in the wild live in murky water where their mottled green and brown pigmented skin provides camouflage from predators

(Reed, 2005; Tinsley, 2010). Diurnal visual predators constitute a significant threat (Baird, 1983), suggesting that camouflage is an important survival mechanism for the species. In contrast to conditions in the wild, *X. laevis* in laboratories are housed in clear water that is regularly (if not continuously) cleaned to maintain optimal frog health and allow visual inspection (Reed, 2005; Green, 2010). However increased water clarity removes a layer of camouflage resulting in potential exposure to predators. Refinement of tank background colour may in part reduce this problem and darkened or opaque tank sides/floors have been suggested as better replicating wild environments and providing a greater sense of security (Council of Europe, 2004). Despite this, a recent survey found that 76 % of laboratories housing *X. laevis* used clear or white tanks, 11 % used tinted tanks and 13 % used black/other dark coloured tanks (unpublished survey, 92 respondents, multiple responses permitted).

Much work has examined the effect of tank colour on aquatic animals, particularly for the aquaculture of fish (e.g. Downing and Litvak, 1999; Karakatsouli et al., 2015) and crustaceans (e.g. Ikhwanuddin et al., 2012; Maciel and Valenti, 2014). Research investigating the effect of background colour on amphibians is more limited, despite crypsis camouflage requirements being important for species that exhibit a background choice (Jonnalagadda et al., 1993; Garcia and Sih, 2003; Wente and Phillips, 2005) or alter their skin colour to match the background (Garcia and Sih, 2003; Segev, 2009). There is no direct empirical evidence of the welfare impacts of tank background on *X. laevis*. Short-term trends for increased growth have been observed in juvenile *X. laevis* housed in black compared to white tanks (Hilken et al., 1995). Juveniles of this species also display a preference for a black background immediately following metamorphosis (Moriya et al., 1996), coinciding with an increase in skin pigmentation (Leadley Brown, 1970). However, as *X. laevis* are long-lived (15-20 years in captivity; Chum et al., 2013) it remains important to understand the welfare impacts of background colour on adults.

Here the impact of an ecologically relevant (black) or a non-ecologically relevant (white) tank background on adult *X. laevis* was investigated. Responses to cage/tank colour in other species have been observed through changes in glucocorticoids (Barcellos et al., 2009; Banan et al., 2013), skin carotenoids (Höglund et al., 2002; Eslamloo et al., 2015), body mass (Sherwin and Glen, 2003),

morphology (Cobcroft et al., 2012), and behaviour (Höglund et al., 2002). In a previous study changes in water-borne corticosterone, behaviour and body mass indicative of stress were observed following transportation of *X. laevis* (Holmes et al., Submitted 2016). This approach is replicated here by comparing adult *X. laevis* responses to housing with either a white background characteristic of that found in laboratory conditions or a black background representative of more naturalistic conditions.

2 Materials and Methods

2.1 Subjects and Housing

Subjects were wild-type *X. laevis* purchased from the European Xenopus Resource Centre (EXRC, University of Portsmouth) and housed at the University of Chester. Housing parameters were established from Reed (2005), Green (2010) and EXRC (A. Jafkins, personal communication). *X. laevis* were housed in single-sex groups of five individuals per glass tank (584 mm x 431 mm x 305 mm, Clearseal) in dechlorinated mains water at a depth of 140 mm with a temperature range 20-23 °C (air temperature 23-25 °C). Water quality was maintained by Hamburg Matten-style biological filtration and partial water changes (~30 %) three times a week. Water quality was checked weekly for pH (6.4-7.6), nitrates (<20 mg/l), nitrites (<5 mg/l) and ammonia (<0.5 mg/l) using a dipstick water testing kit (King British). *X. laevis* were housed under a 12:12 light:dark cycle and fed 2.3 mm Royale Horizon Trout Pellets (Skretting) three times a week. Home tanks were provided with black PVC tubing (2 per tank, 140 mm x 118 mm x 50 mm, Floplast) and terracotta pots (1 per tank, 135 mm x 75 mm) as environmental enrichment (Reed, 2005; Green, 2010). Photographs of *X. laevis* markings were used for individual identification (Reed, 2005).

2.2 Tank Background

In order to investigate the effect of tank background, *X. laevis* (n = 36, 20 females, 16 males) were weighed and housed individually in experimental tanks (254 mm x 203 mm x 208 mm, Clearseal) at 09:00 with either black (weed control fabric, Verve) or white (Evolution Value A4 paper) backgrounds in a repeated measures design. Backgrounds were attached to the outside of experimental tanks and covered the floor, two long walls and the rear short wall, allowing for observation of frogs through the front short wall. After 48 hours the frogs were sampled for

corticosterone (see below), weighed and returned to home tanks. One week later the frogs were placed into the same tanks with the reverse background colour and after 48 hours the sampling process was repeated. Background colour presentation order was randomised but balanced (18 frogs exposed to each background colour first).

2.3 Behaviour

Behaviour was observed during each trial over two 30 minute periods: immediately following placement of frogs (Entry) into experimental tanks and immediately before removal from experimental tanks (prior to hormone sampling - Exit). Behaviour was recorded (Handycam® Camcorder, Sony) and footage watched back by an observer blind to the identities of individual frogs and the sampling occasion (it was impossible to be blind to treatment group). Behaviour was quantified using an ethogram for *X. laevis* developed previously (Holmes et al., Submitted 2016; Table 1). Common behaviours were recorded using focal instantaneous time sampling with an interval of 30 seconds and expressed as proportions of the total number of sample points. Short-duration behaviours were recorded using one-zero sampling methods and expressed as proportions of the total number of sampling intervals.

2.4 Hormone Sampling

Total corticosterone (free + conjugated) release rates of *X. laevis* were obtained non-invasively by extracting hormones from the surrounding water using methods modified from Ellis et al. (2004) and validated for *X. laevis* by Holmes et al. (Submitted 2016). Following the second behavioural recording period (Exit) *X. laevis* were placed into individual collection tanks (210 mm x 130 mm x 140 mm; Hagen) containing 1000 ml deionized water (Labwater 1, Purite). After 1 hour frogs were removed from collection tanks, weighed and returned to home tanks. On all sampling occasions two empty water samples were collected to control for background corticosterone. The mean corticosterone titre of these empty samples was subtracted from *X. laevis* samples collected at the same time to get a true measure of the amount of corticosterone excreted by *X. laevis* over 1 hour.

Following collection the water samples were vacuum filtered through filter paper (pore size = 11 µm, Fisherbrand) and cellulose nitrate filter paper (pore size = 0.45 µm, Sartorius). Water samples were

pumped through activated solid phase extraction cartridges (primed with 5 ml HPLC-grade 100 % methanol and 5 ml distilled water; Sep-pak® Plus C18, Waters Ltd.) at 25 ml/min (Ellis *et al*, 2004), washed of impurities with 5 ml distilled water and stored at (-4 °C) until elution.

Corticosteroids (20 °C) were eluted from cartridges into borosilicate glass tubes (16 mm x 100 mm, Fisherbrand) using 4 ml ethyl acetate. The ethyl acetate was evaporated under nitrogen at 37 °C and the samples re-suspended in 500 µl EIA PBS buffer (0.1 g BSA in 100 ml 0.1 M PBS. PBS = 5.42 g $NaH_2PO_4H_2O$, 8.66 g Na_2HPO_4 (anhydrous), 8.7 g NaCl, 1000 ml d.H_2O, pH 7). Samples were vortexed (Multi-Reax, Heidolph) at 1600 rpm for 20 minutes and stored at -4 °C until required.

2.5 Enzyme Immunoassay

Holmes et al. (Submitted 2016) previously validated an enzyme immunoassay (EIA) to quantify *X. laevis* water-borne corticosterone release rates. Briefly, the antibody (CJM006) was diluted 1:16,000 in 0.05 M carbonate buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 1000 ml d.H_2O, pH 9.6), loaded 50 µl/well onto a 96-well Maxisorp Nunc-Immuno microtitre plate (Thermo-Fisher Scientific, UK) and incubated overnight (4 °C).

Plates were washed four times with 1:5 diluted ELISA wash buffer (40 g NaCl, 1 g KCl, 1.2 g KH_2PO_4, 7.2 g Na_2HPO_4, 2.5 ml Tween 20, 1000 ml d.H_2O, pH 7). The plate was loaded with 50 µl/well EIA buffer, followed by either 50 µl/well corticosterone standard or 50 µl/well *X. laevis* sample (diluted 1:2 in EIA buffer), and 50 µl/well of horseradish peroxidase conjugate (1:40,000 in EIA buffer) and left to incubate for 3 hours in darkness. The plate was washed as before and 100 µl/well EIA substrate was added (12.5 ml Citrate buffer, 125 µl EIA ABTS, 40 µl H_2O_2. Citrate buffer – 9.61 g citric acid (anhydrous), 1000 ml d.H_2O, pH 4. EIA ABTS – 0.329 g ABTS, 15 ml d.H_2O, pH 6. H_2O_2 – 2%w/v, 500 µl H_2O_2, 7.5 ml, d.H_2O). The plate was left to incubate in darkness until the blank wells reached an optical density of 1.0. The plate was read at 405 nm using a microplate reader (MRX II, Dynex Technologies; Revelation, Version 4.22). Samples were run in quadruplicate, and re-run along with other samples from the same individual if any coefficients of variance (CV) were above 5 %. Both samples from the same individual collected from the two conditions were always run on the same plate.

2.6 Statistical Analyses

All statistical analysis was carried out using SPSS version 21.0. Corticosterone release rates were expressed as pg/hr. Corticosterone samples were excluded if water samples were spilt or females laid eggs during individual housing as reproduction impacts corticosterone output in amphibians (Moore and Jessop, 2003). Corticosterone data was log transformed to meet parametric assumptions. Differences between Entry and Exit body mass recordings were analysed for each background colour using Wilcoxon Signed Ranks tests. Change in body mass over 48 hours was recorded as mass at start minus mass at end of each trial. Repeated measures general linear models (GLM) were used to separately compare corticosterone and change in body mass following housing with a black or white tank background, with sex and background presentation order as between-subject factors.

Due to camera availability the behaviour of two females and two males was not recorded. Entry Walling behaviour was arcsine transformed to and analysed in the same manner as corticosterone and change in body mass. As the remaining behavioural observations did not meet parametric assumptions behaviour ratios were calculated (behaviour in black/white) and Mann-Whitney U tests checked for differences in behaviour ratios between the sexes and between background presentation order groups. Proportions of behaviours in the black and white backgrounds were then compared using Wilcoxon Signed Ranks tests. To investigate any change in behaviour, Entry and Exit behaviours for the first trial only were compared using Wilcoxon Signed Ranks tests (where a difference in behaviour was observed between the black and white backgrounds these trials were spilt by background type). As Stationary was the only other available Common behaviour it was inferred to have the same (but opposite) result to Swimming and analysis was not performed on Stationary.

2.7 Ethics

All work was carried out in consultation with the Home Office, following University of Chester Research Guidelines and under approval from the University of Chester Faculty Research Ethics committee.

3 Results

3.1 Corticosterone

A significant interaction between background type and sex in corticosterone release rates was observed ($F_{(1,28)}$ = 6.742, p = 0.015), and overall female *X. laevis* released significantly higher levels of corticosterone than males ($F_{(1,28)}$ = 10.721, p = 0.003). Separate analyses by sex revealed that female *X. laevis* exhibited higher corticosterone release rates when housed on a white background compared to a black background ($F_{(1,15)}$ = 4.707, p = 0.047; Figure 1) however there was no difference in corticosterone release rates with background type in males ($F_{(1,13)}$ = 2.299, p = 0.153; Figure 1). Background presentation order had no effect on corticosterone release rates in any analysis (p > 0.05).

3.2 Body Mass

There was a reduction in *X. laevis* body mass over the course of each background trial (White: z = -5.308, p < 0.001; Black: z = -4.575, p < 0.001; Figure 2), however a greater amount of body mass was lost when frogs were on a white background compared to on a black background ($F_{(1,29)}$ = 5.914, p = 0.021; Figure 2). There was no effect of sex or presentation order on body mass change and no significant interactions (all p > 0.05).

3.3 Behaviour

On Entry to the experimental tanks no difference in the proportion of Swimming behaviour was observed between the background types (z = -0.435, p = 0.673; Figure 3a). However on Exit from the experimental tanks a greater proportion of Swimming behaviour was observed in frogs housed with a white background compared to a black background (z = -2.028, p = 0.042; Figure 3a). There was no difference in proportion of Swimming behaviour between Entry and Exit observations when frogs were housed on a white background (z = -0.245, p = 0.826), however Swimming decreased between Entry and Exit when frogs were housed on a black background (z = -2.244, p = 0.023; Figure 3a).

On Entry to the experimental tanks more Walling behaviour was performed by frogs when housed on a white background compared to a black background ($F_{(1,29)}$ = 4.523, p = 0.042; Figure 3b). On Exit from the experimental tanks there was no difference in the proportion of Walling behaviour observed between the background types (z = -0.796, p = 0.438; Figure 3b). In both background types the

proportion of Walling behaviour observed decreased between Entry and Exit observations (White: $z = -2.354$, $p = 0.017$. Black: $z = -2.613$, $p = 0.007$; Figure 3b).

No differences in the proportion of Breathing were observed either between background types or Entry and Exit observations (all $p > 0.05$). No difference in the proportion of Bubbles was observed between white and black background types on either sampling occasion, however the proportion of Bubbles observed was lower on Exit than on Entry ($z = -3.030$, $p = 0.001$). Too few instances of Burst Breathing ($n = 0$) and Sloughing ($n = 3$) were observed for analysis. No differences between the sexes or presentation order were observed for any behaviour (all $p > 0.05$).

4 Discussion

The behavioural and physiological responses of adult *X. laevis* differed with tank background colour, with tanks with a non-ecologically relevant background (white) leading to higher corticosterone release rates in females but not males. Measures of glucocorticoids are best supported by other physiological and/or behavioural data (Otovic and Hutchinson, 2015) and greater body mass loss and increased active behaviours in both sexes were also observed in white background tanks compared to black background tanks. Changes in glucocorticoids, behaviour and body condition may all be indicators of stress in other species (Broom, 1991) and similar increases in corticosterone release rates and active behaviours, as well as decreases in body mass, have been previously observed in *X. laevis* following a transportation stressor (Holmes et al., Submitted 2016). Body condition checks of frogs following the trials revealed that a small proportion developed small rubs or sores on the tips of their snouts. Whilst data was not obtained regarding frequencies of sores in different tank backgrounds, the sores appeared to be as a result of repeated swimming against the tank walls (Walling), a greater proportion of which occurred in tanks with white backgrounds. Following the experimental trials, body mass returned to normal and the sores quickly healed and did not return, indicating no long term health or welfare implications.

The results presented here complement studies of *X. laevis* development where a preference for black over white backgrounds was shown in metamorphosing tadpoles (Moriya et al., 1996) and short-term

growth of juveniles was higher in black compared to white tanks (Hilken et al., 1995).Together this suggests that for adult *X. laevis,* housing with a non-ecologically relevant (white) background may cause more behavioural and physiological changes indicative of a stress response compared to housing with an ecologically relevant (black) background. This finding is particularly important given that *X. laevis* are long lived in captivity and may be housed in the same environment for many years (Chum et al., 2013), extending the impacts that refinements to captive housing can have on individual welfare.

Refined background colour may reduce perceived predation risk in species that rely on crypsis camouflage (Garcia and Sih, 2003). The mottled green and brown pigmented skin of *X. laevis* appears in stark contrast against a white background and as a result the frogs may feel more exposed to visual predators which are a significant threat in the wild (Baird, 1983; Reed, 2005). During the light phase captive *X. laevis* choose to rest in locations that minimise their exposure to predation (Archard, 2013). Therefore the increased activity in the white background condition may be a result of the frogs attempting to find cover due to an increased perception of own exposure. *X. laevis* are able to change the lightness of their skin in response to light (Roubos, 1997), potentially negating any negative crypsis effects of housing on a white background over the long-term. However, *X. laevis* housed for several months with a light background remain clearly visible despite skin colour adjustment (Holmes, personal observation). Importantly, cryptic behaviour may also be independent of own body colour, as in northern leopard frogs (*Rana pipiens*) that display a similar preference for background colour regardless of own morph colour (Croshaw, 2005), and therefore the results observed here may hold true regardless of skin colour change.

Background colour may also influence perception of the internal architecture of the captive environment and in fish species refinement of tank colour can reduce incidence of injury or mortality resulting from tank wall collision (Okada et al., 2015) or increase juvenile predator avoidance (Jones and Kaiser, 2005). *X. laevis* are thought to have limited vision in water (Chum et al., 2013) and white walls may have made tank boundaries harder to identify than black walls. Levels of phototaxis behaviour (moving towards or away from a light source) may also be affected by tank background

colour, a suggested explanation for high levels of walling behaviour and jaw malformation observed in striped trumpeter (*Latris lineata*) larvae housed in white tanks (Cobcroft et al., 2012). *X. laevis* are known to move away from light sources (Karplus et al., 1981; Hilken et al., 1995) and the white tanks may have prompted increased attempts to avoid the light background. An increase in locomotion behaviours within the white tanks as a result of one or more of these reasons may have subsequently caused the increased corticosterone release rates and decreased body mass.

The specific optimal housing background colour is likely to be influenced by a species' own life history requirements and visual ability (e.g. Duray et al., 1996; Tamazouzt et al., 2000; Strand et al., 2007; Ullmann et al., 2011; Cobcroft et al., 2012; Rahnama et al., 2015). A lighter tank background colour may increase food item perception leading to improved feeding ability (El-Sayed and El-Ghobashy, 2011) or reduced negative consequences of competition for food (Sykes et al., 2011). *X. laevis* hunt using a lateral line system (Chum et al., 2013). A decrease in food:background contrast due to housing refinements is therefore unlikely to negatively influence feeding ability or competition in this species. In species where dominance status is signposted through body colour, lower levels of aggression can be achieved through tank background colour modification (Höglund et al., 2002). To date, however, there is no record of body colour being used as a signal in *X. laevis* and as a result tank background refinement is unlikely to negatively affect welfare for this reason. Life stage is also an important consideration for background colour refinement, particularly in species such as *X. laevis* where dramatic morphological changes occur during development (Moriya et al., 1996). A change in housing conditions with metamorphosis may therefore be important in this species.

4.1 Scientific Research Implications

Improved welfare may also increase the effectiveness of research involving *X. laevis*. A large proportion of research using *X. laevis* requires the production of oocytes (Schultz and Dawson, 2003), however stress is known to affect reproduction in a number of species (Whirledge and Cidlowski, 2013). *X. laevis* oocyte quantity and quality may be improved by housing with environmental enrichment (Harr et al., 2008) and the results found here suggest that refined tank background colour may similarly be used to improve oocyte production and consequentially scientific research.

Welfare improvements may not always be mirrored by easier laboratory protocols (Archard, 2012). Individual identification of *X. laevis* can be achieved by observation of their skin patterns (Schultz and Dawson, 2003). As the patterns remain constant and visible (Schultz and Dawson, 2003) a change in tank background colour does not affect identification and *X. laevis* housed for several months with black backgrounds remain easy to distinguish (personal observation). A light background enables the easy recognition and cleaning of grime (Reed, 2005) however thorough filtration and cleaning protocols negate this issue. A dark background or opaque tank walls may also make it harder to perform adequate health checks, however in the current study the clear front panel in the experimental tanks allowed for easy observation of both the tank and frogs. Tinted tanks may also provide an alternative, enabling observation without disturbance. Translucent red laboratory mouse houses are perceived as close to opaque by the mice, providing them with cover but allowing for undisturbed visual inspection (Soerensen et al., 2009). In a similar manner, a recent study on the closely related species *Xenopus tropicalis* found that translucent red may be employed as an overhead cover (Cooke and Giroux, In prep). Further tests are required to examine whether translucent red tank walls might function in the same manner for adult *X. laevis*.

4.2 Conclusion

The results presented here show for the first time that tank background colour is an important aspect of adult *X. laevis* captive housing and welfare. Non-ecologically relevant (white) backgrounds produced higher water-borne corticosterone release rates, a greater proportion of atypical locomotion behaviour and a greater drop in body mass compared to ecologically relevant (black) backgrounds. These findings are crucial for the welfare of this model species and its effective use in research.

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Table 1: Ethogram for recording *X. laevis* behaviour

Common Behaviours	
Stationary	Motionless within the tank, either fully submerged or with part of body breaking water surface.
Swimming	Moving through the water using slow, measured hind-limb kicks; often accompanied by slow paddling motions with forelimbs.
Short-duration Behaviours	
Breathing	Slow movement to the surface, movement visible in throat area, and return to full submersion.
Bust Breathing	Dart to the surface, releasing air bubbles, gulps air and re-submerges; a continuous movement in under 2 seconds.
Bubbles	Release of an air bubble(s) whilst remaining fully submerged.
Sloughing	Rubbing sections of body or violently kicking to remove sections of skin; grooming top of head with forelimbs; skin often consumed immediately.
Walling	Fast swimming back and forwards along a tank wall; rapid rear limb kicks; scrabbling at tank walls with forelimbs; snout against tank wall.

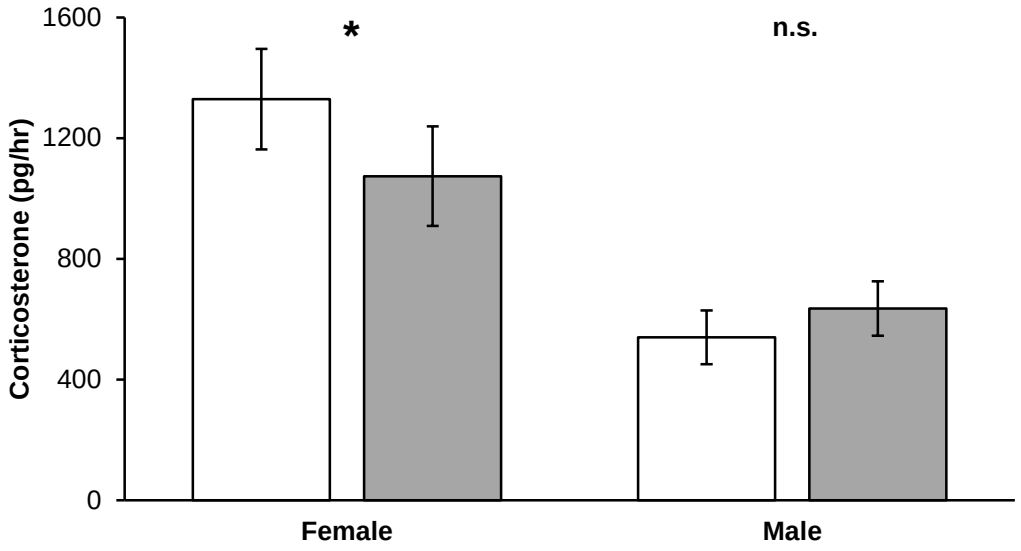

Figure 1: Corticosterone release rates (pg/hr, mean ± s.e.) of female and male *X. laevis* when housed for 48 hours in tanks with either a white (open bars) or a black background (grey bars). Significant differences ($p < 0.05$) indicated by *.

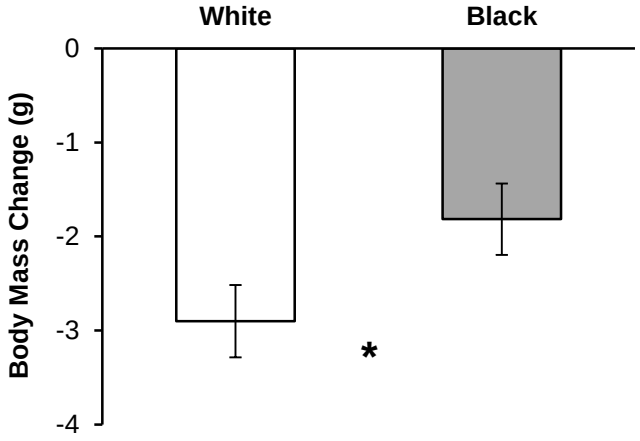

Figure 2: Decrease in *X. laevis* body mass (g, mean ± s.e.) following housing in tanks with either a white (open bars) or black (grey bars) background for 48 hours. Significant differences ($p < 0.05$) indicated by *.

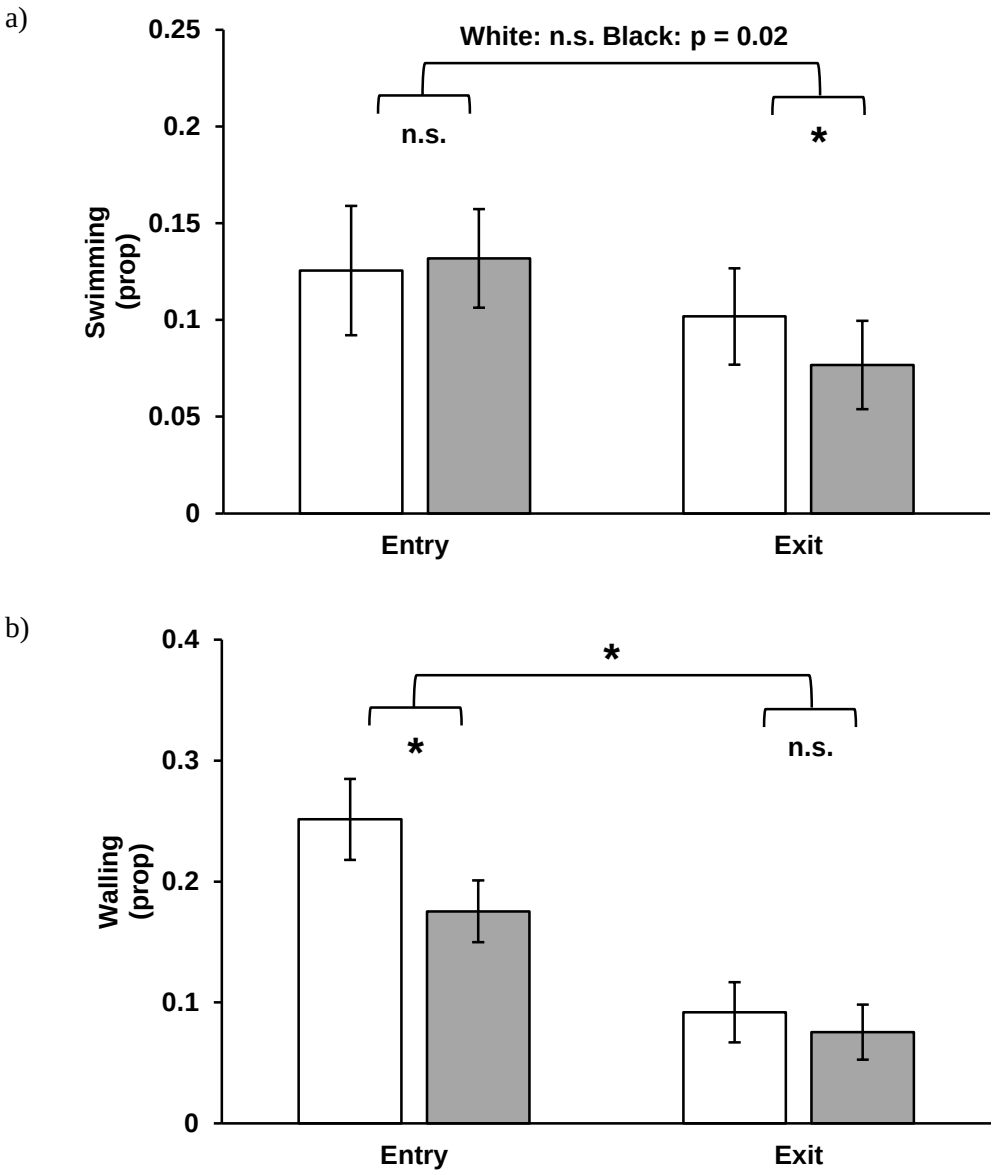

Figure 3: Proportion of time (mean ± s.e.) spent performing a) Swimming or b) Walling behaviour by *X. laevis* on Entry to or Exit from housed in tanks with a white (open bars) or black (grey bars) background. Significant differences ($p < 0.05$) indicated by *.